SOME OBSERVATIONS ON THE FRAGILITY OF THE RED BLOOD CORPUSCLES, WITH SPECIAL RELATION TO THE STORAGE OF BLOOD FOR TRANSFUSION.

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The collection of blood for transfusion purposes, and its storage in a refrigerator until required, for a period of two or three weeks has been proved practicable, though is still, to an extent, experimental.

The work of Bagdassarov[1], and others in Russia, claims precedence, as a source of blood they relied largely on cadaver, while Goodall[2], Anderson, Altimas and Mc.Phail, and Crodberg and Carey[3], in the United States investigated placental blood as a source of supply.

Cadaver blood, probably on ethical grounds, does not appear to have been used in this country, and reports on its value during the Spanish Civil War are most unfavourable, due mainly to the difficulties arising in the collection of it under wartime conditions.

Placental blood has been used, and Page, Seager and Wadd[4] stress its undoubted advantages, but Howkins and Brewer[5] found that the very small quantities obtained, and the considerable chances of contamination during collection, render this means of obtaining blood for transfusion purposes, inadvisable. The collection of blood from healthy donors at their convenience has found favour, and from its many advantages, has quickly become an established practice. Articles published by Elliott, Macfarlane and Vaughan[6], Boland, Craig and Jacobs[7], and Biddle and Langley[8] early in 1939, described the successful collectiom storage and transfusion of such blood.

The very wide experiences gained in wartime in Spain were published by Jorda[9], who administered very large quantities of pooled stored blood to war casualties with excellent results. Vaughan[10] reviewed the whole position at that time, (May 1939), and several questions, the choice of anticoagulant, choice of donor, and storage conditions required further investigation.

Harrington and Miles[11] stored the blood of volunteers under varying conditions, and advanced the subject from the experimental angle considerably.

A series of experiments were embarked upon to try to assess the relative merits of the various factors in regard to blood storage, by the collection of a large number of specimens from different donors, and their observation during storage.

Storage took place in a refrigerator whose optimum temperature was 4 Deg. Centigrade, plus/minus 1 Deg. Centigrade. The question of optimum temperature was studied by Bagdassarov who found that freezing caused massive haemolysis, and temperatures above 0 Deg. up to 7 Deg. Centigrade were suitable.

The method used was to collect blood samples, generally 10c.cs. by syringe from the veins of donors, to mix the blood with the chosen anticoagulant, and store the sample in a glass stoppered bottle of 30 c.cs. capacity. All aseptic precautions were carefully observed, and if, in any specimen, gross contamination was suspected, that specimen was rejected.

Observations were made under the following three classes;

a. Choice of Blood Donor.

b. Choice of Anticoagulant.

c. Various methods of Storage.

At first, the criterion of results was estimated by actual enumeration of the red corpuscles present by haemocytometer, carried out at frequent intervals, care being taken to carefully mix the specimen first; and the results obtained were interesting, but the formation of a small loose clot upset these figures. All specimens which clotted in any way were therefore rejected, though such have been used successfully for transfusion after filtration. Fig.1. shows the findings for three types of anti-coagulants, showing maximum and minimum curves for each. These samples were all collected from healthy adult males, and no special precautions as regards oxygenation etc. were taken. Anticoagulants used were numerous; 1-5% Sodium Citrate alone, in normal saline, with Glucose in various strengths, I.H.T. recommended by Bagdassarov and others, and a similar solution omitting the Potassium Chloride and Magnesium Sulphate. The criteria of a suitable anticoagulant depends on two factors, 1. the absence of coagulation from the blood specimen, and 11. Minimal haemolysis when mixed with the blood.

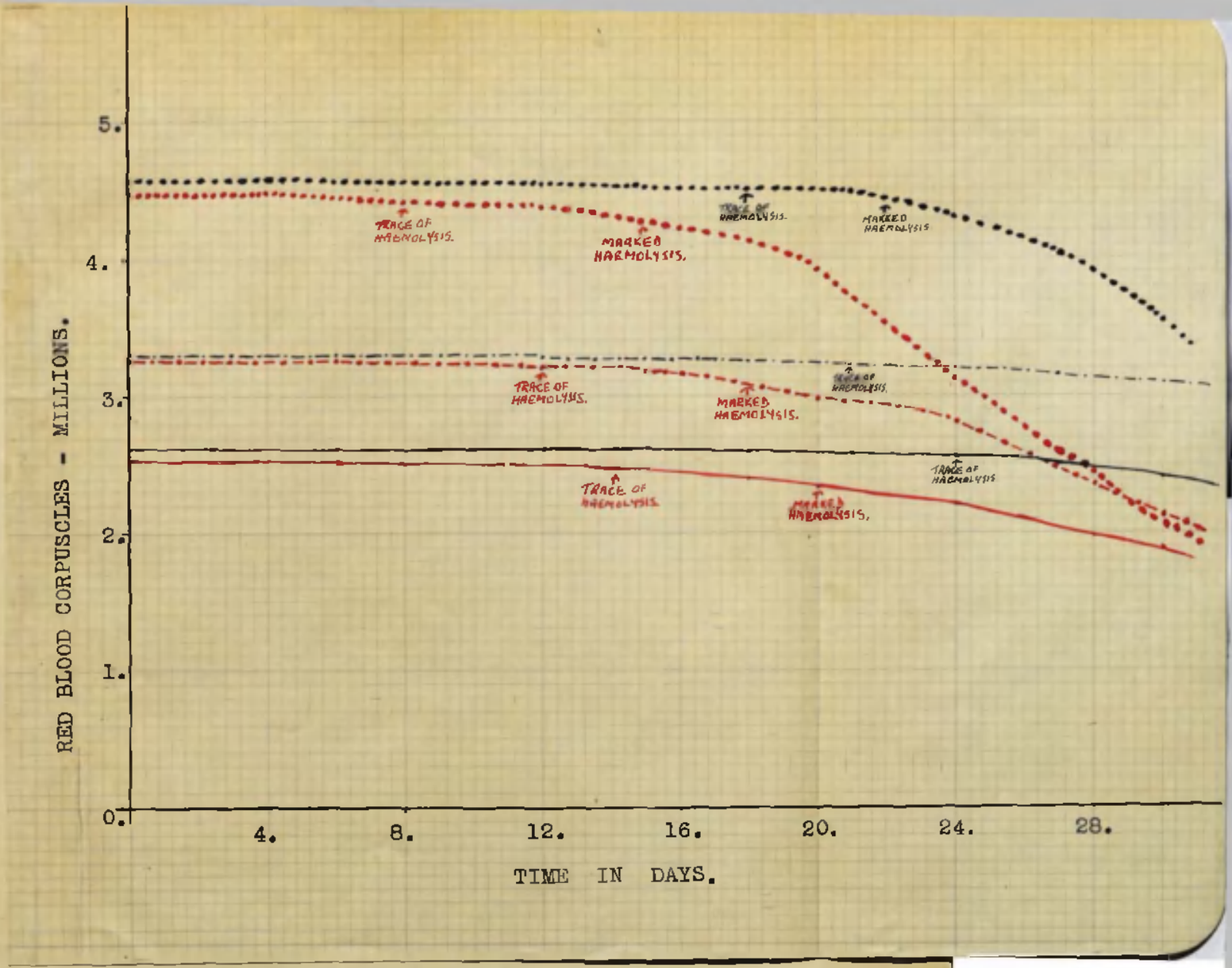


FIG 1.

..... Minimum) Red Count Curve from Blood with 1-9
..... Maximum) Dilution with 3.8% Sodium Citrate Soln.

-.-.-.-.- Minimum) Red Count Curve from blood with 1-3
-.-.-.-.- Maximum) Dilution with Glucose Citrate Saline.

——— Minimum) Red Count Curve from blood with 1-2
——— Maximum) Dilution with I.H.T.

The three anticoagulants mentioned in Fig 1. are typical of all employed; the first is typical of the fairly concentrated citrate solutions, generally used in a dilution of about 10%, and as is shown, compares unfavourably with the others. When used in greater quantities up to 50%, only very slight improvement in such a curve was present, and with higher concentrations, considerable haemolysis appeared after as little as 24 hours. The second anticoagulant is that recommended by Harrington and Miles, Sodium Citrate 1.05%, Glucose 0.5%, Sodium Chloride 0.9%,

omitting the Sulphanilamide recommended by them, and used in in a 1-3 dilution. The results obtained are similar to most of the anticoagulants employed, with dilutions of 25-30%, and for a period up to 14 days the results were excellent. Much less haemolysis occurred than in specimens with smaller quantities of more concentrated Citrate solutions and they were only slightly bettered by I,H.T. stored specimens. These are also shown on Fig. 1. and in this investigation, it gave slightly but consistently better results than a similar dilution of Saline Citrate, with or without added Glucose, or any other anticoagulant.

An attempt was now made to air condition some specimens. Similar samples were taken, mixed with the same amount of the same anticoagulant, and stored;

a. After thorough Oxygenation.

b. After Saturation with Carbon Dioxide.

c. In a bottle from which air was excluded by overfilling before stoppering.

The "air conditioning" was carried out by simply bubbling the gas into the sample through a sterile capillary tube, and this process was repeated each time the specimens were opened for examination. Fig. 2. shows the results of this investigation, and demonstrates a definite advantage of storage of the oxygenated samples over the Carbon Dioxide saturated samples, and a less but definite advantage over the completely filled bottles. This finding was confirmed using several anticoagulants, only these of two of them used before are shown.

Oxygenation is practised by Jorda, using compressed air in part of his storage canister, and he states that the Russian school have successfully used 1% Peroxide of Hydrogen to ensure this, while Harrington and Miles state that oxygenation, is or rather, storage in compressed oxygen is of no value. These experiments suggest that corpuscles containing oxyhaemoglobin store much better than these containing the Carbon Dioxide Compound, by being much less liable to early haemolysis.

This recalled the work of Whitby and Hynes,[12] on the fragility of red blood corpuscles, and led to a further series of similar experiments on the estimation of the fragility of the stored corpuscles.

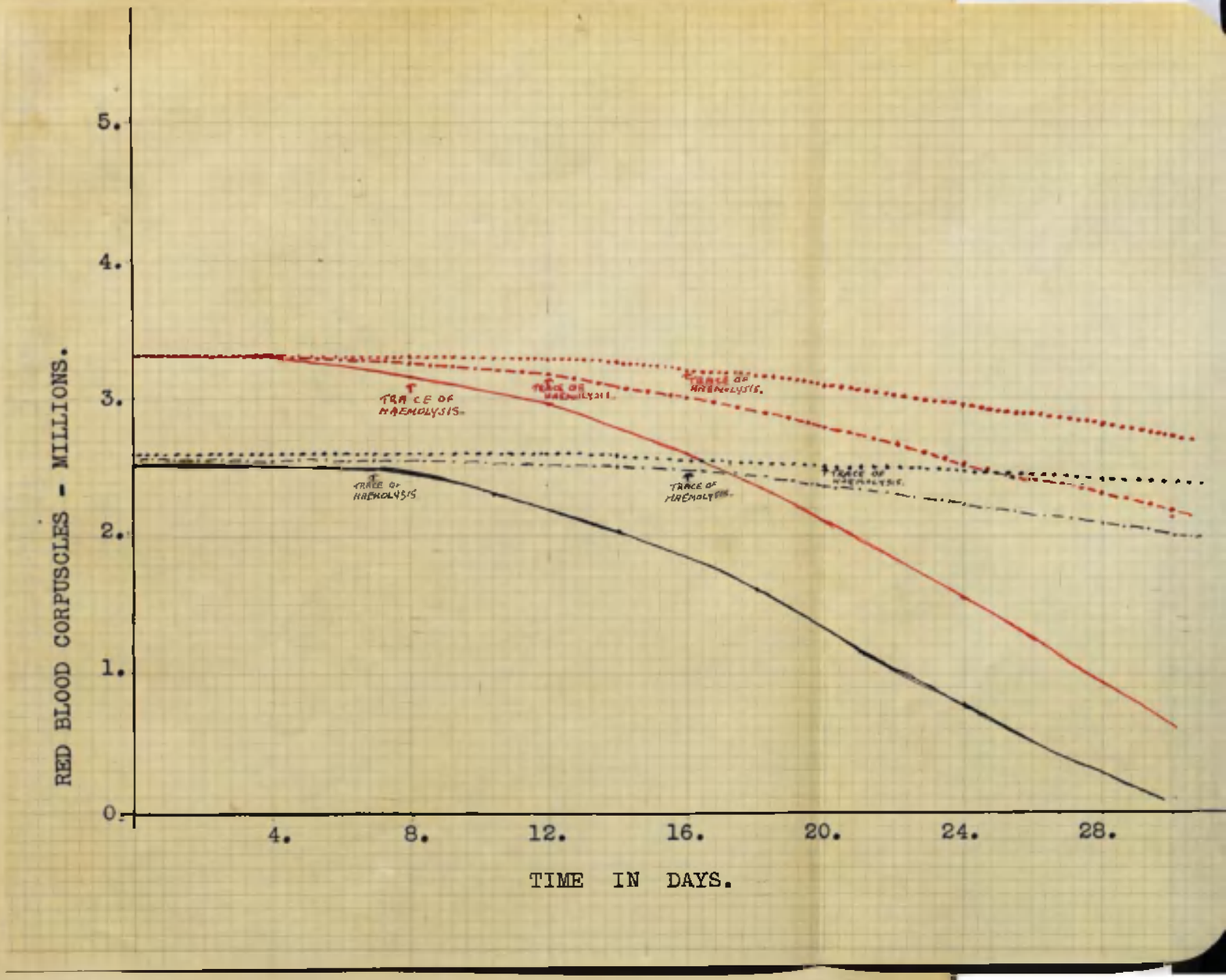


Fig. 2.

..... Blood stored in Glucose Citrate Saline plus Oxygen.

——— Blood stored in Glucose Citrate Saline plus CO2.

-.-.-.-.- Blood stored in Glucose Citrate Saline-filled bottle.

..... Blood stored in I.H.T. plus Oxygen.

——— Blood stored in I.H.T. plus Carbon Dioxide.

-.-.-.-.- Blood stored in I.H.T. completely filled bottle.

It is worthy of note that the three specimens shown in red were obtained from the same donor at the same time.

In the estimation of the fragility of these specimens, the usual clinical method was adopted, but no attempt was made to wash the corpuscles. A drop of the stored blood after mixture, was dropped into tubes containing hypotonic saline solutions, allowed to stand for an hour, and then the supernatant fluid

was examined spectroscopically for traces of haemoglobin in solution. The solutions with a difference of 0.05% were replaced by graded solutions differing by 0.03% checked by the titration with Silver Nitrate mentioned by Whitby and Hynes, as this gave more detailed information. An attempt was made later to still further reduce the difference in concentrations by half, but the differences were then so slight that no greater accuracy was achieved. The curves obtained are therefore of the step ladder type, but none the less informative. It must also be stressed that when haemolysis occurred in a sample fragility could no longer be estimated by this method.

Fig. 3. shows this curve for three typical samples using the same anticoagulants as before. All were oxygenated.

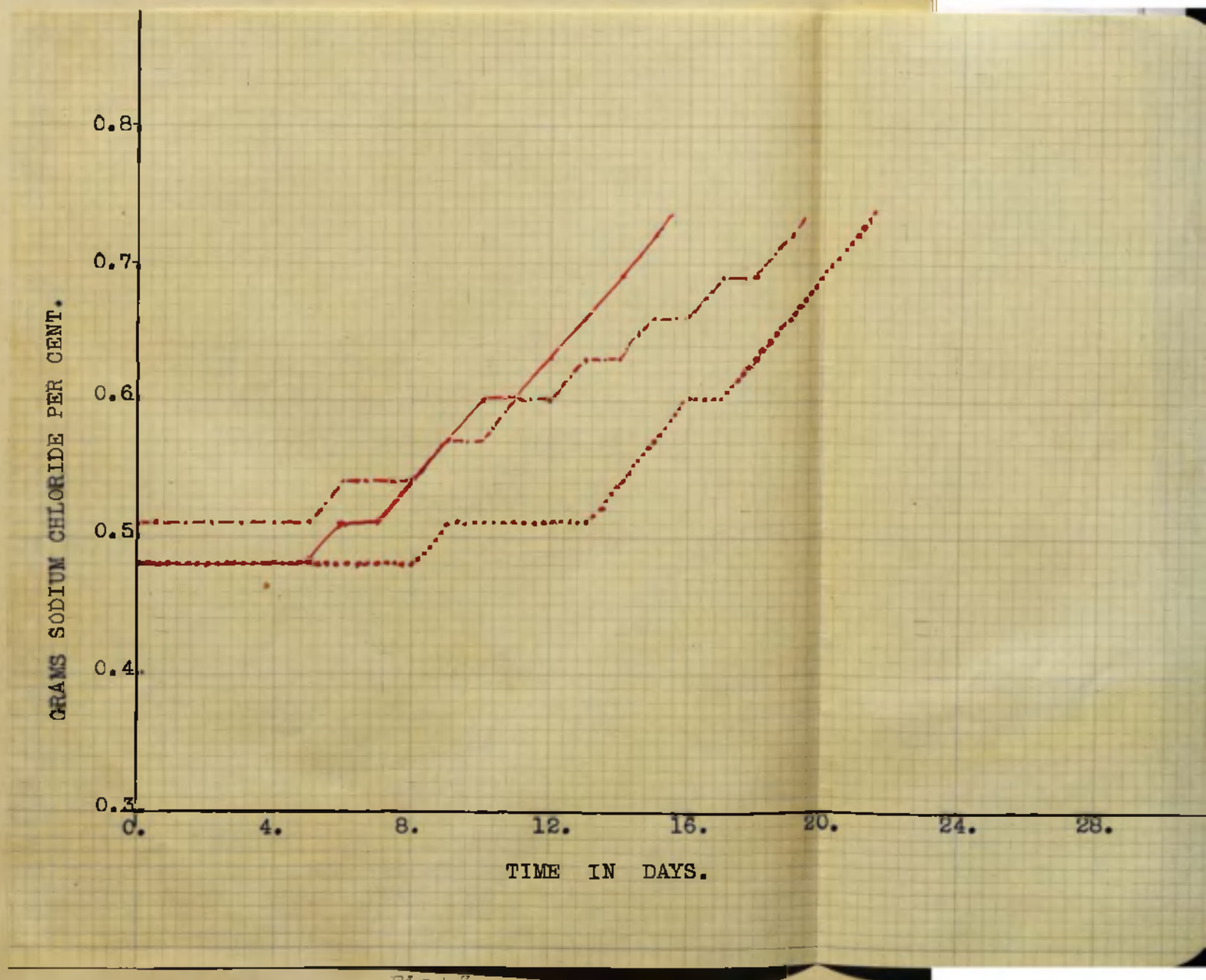


Fig. 3.

——— Blood stored in 3.8% Citrate 1-9.
—·—·— Blood stored in Glucose Citrate Saline 1-3.
····· Blood stored in I.H.T. 1-2.

Fig. 3 showed a gradual decrease in the resistence of the stored red blood corpuscles to the haemolysing action of hypotonic solutions of Sodium Chloride, a progressive decrease in saline fragility. This decrease appeared rather slower in the Glucose Citrate Saline preserved specimens than in the others, while I.H.T. preserved specimens seemed to have the greatest resistence for the first fortnight of storage. The only further observations along these lines are shown in Figs. 4 and 5, which show the fragility curves for specimens similar to those whose cells were enumerated in Fig. 2.

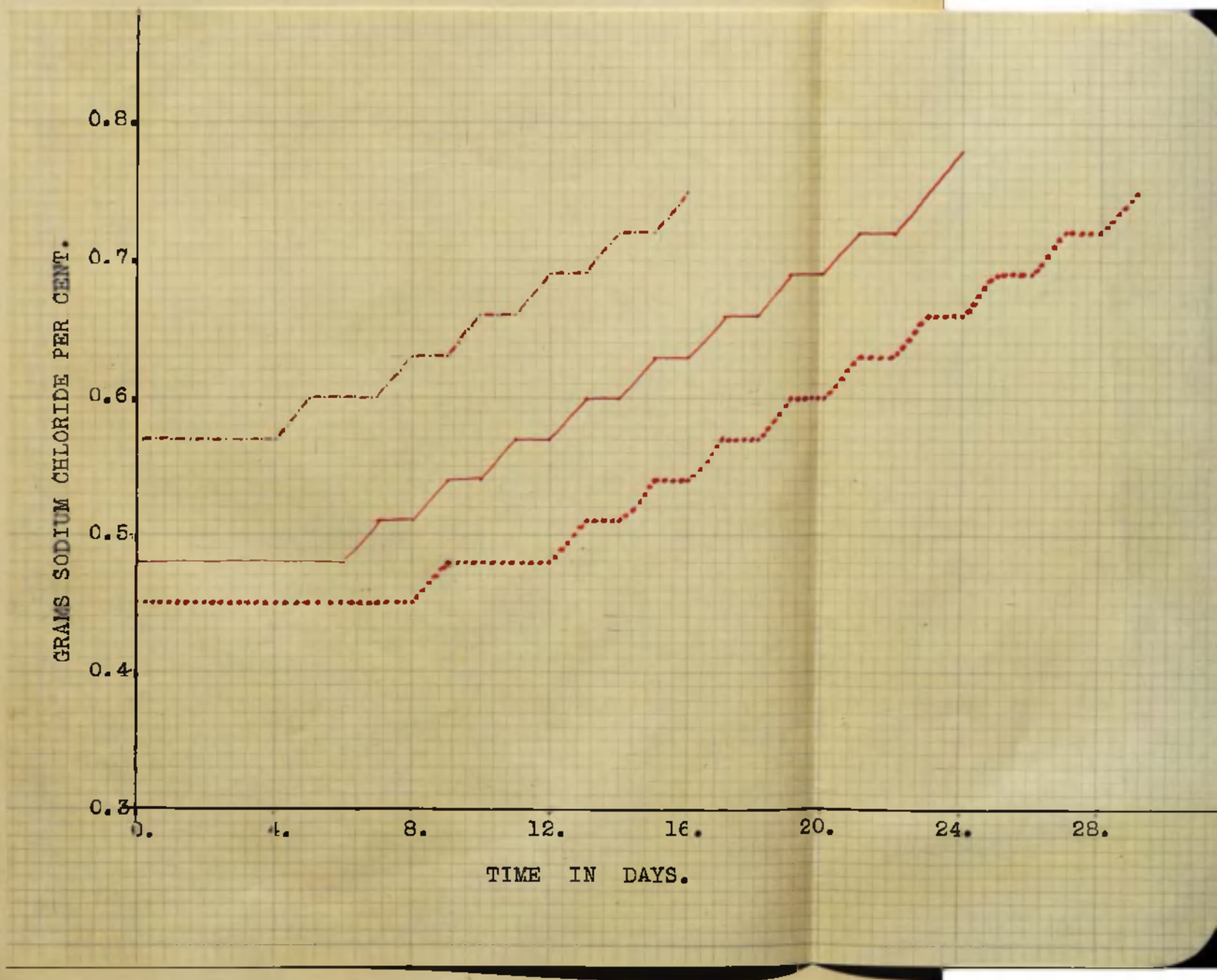


Fig. 4.

Fragility Curve of blood in Glucose Saline Citrate

..... Plus Oxygen.

-.-.-.- Plus Carbon Dioxide.

_____ In Airfree Container.

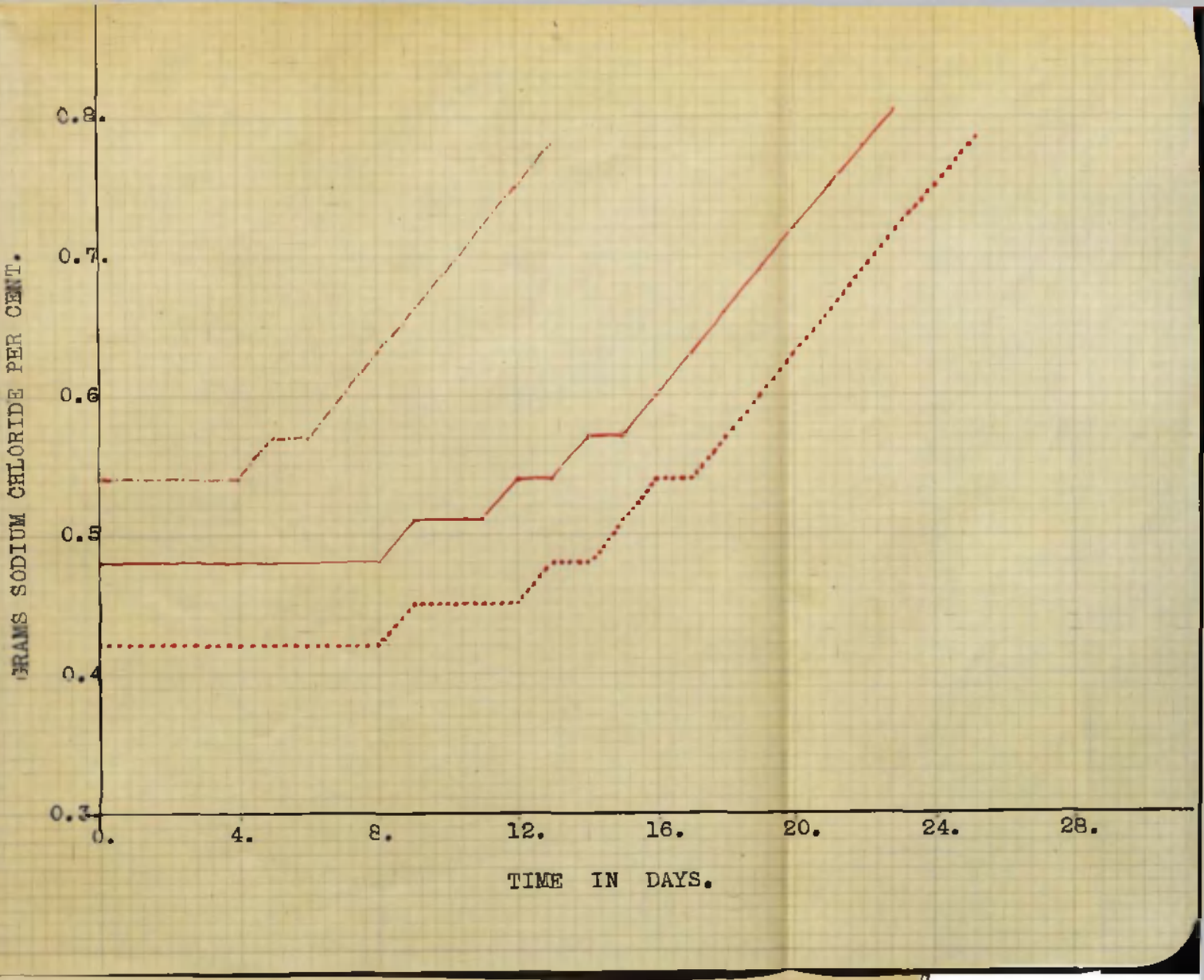


Fig.5.

Blood Stored in I.H.T. 1-2.

..... Plus Oxygen.

-.-.-.-.- Plus Carbon Dioxide.

——— In Airfree Container.

Comparison of Figs. 4 and 5 strengthens the suggestion that the rate of decrease of fragility is less in the glucose Citrate saline, and this was proved by observation of two identical specimens, from one of which, the glucose was witheld. Their fragility curve is shown in Fig. 6, and at the same time two specimens, one mixed with I.H.T. and a similar mixed in I.H.T. without Magnesium Sulphate and Potassium Chloride were compared.

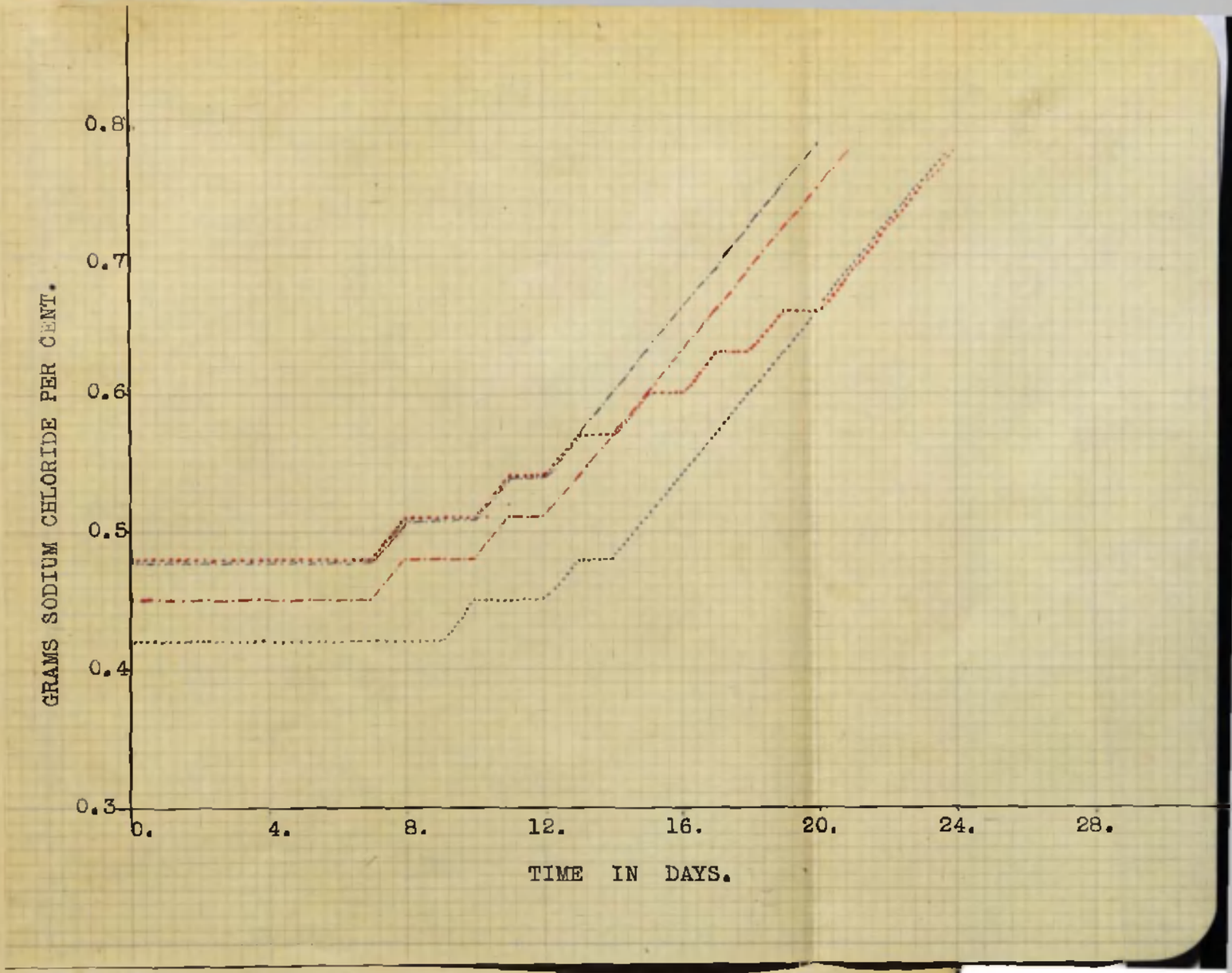


Fig. 6.

..... Blood with1-3 Glucose Citrate Saline.

-.-.-.-.- Blood with 1-3 Citrate 1.05%, Sodium Chloride 0.9%.

..... Blood with I.H.T. 1-2.

-.-.-.-.- Blood with Citrate 0.5%, Sodium Chloride 0.7%.1-2.

All these specimens were oxygenated.

From these curves it appears that the presence of glucose decelerates the increase in fragility by its presence, and the Magnesium Sulphate and Potassium Chloride in I.H.T. actually increase the fragility of the stored red cells. Further experiments showed that the Magnesium Sulphate had this effect, and that its onset was immediate after mixing. Experiments with I.H.T. with added glucose, however, gave figures similar to I.H.T. alone.

To obtain more precise information about the variability of the fragility of the erythrocytes under storage conditions, recourse was now made to the Quantitative method of estimation devised by Whitby and Hynes, and the following curves show the findings obtained. Fig. 7. shows the fragility of a healthy male donor aged 30. R.B.C. 5,180,000 per cu mm., Hb. 100%, the estimations being made one to four hours after collection, Figs. 8, 9, and10 show the behaviour of these same samples after storage, further curves being taken at four day intervals.

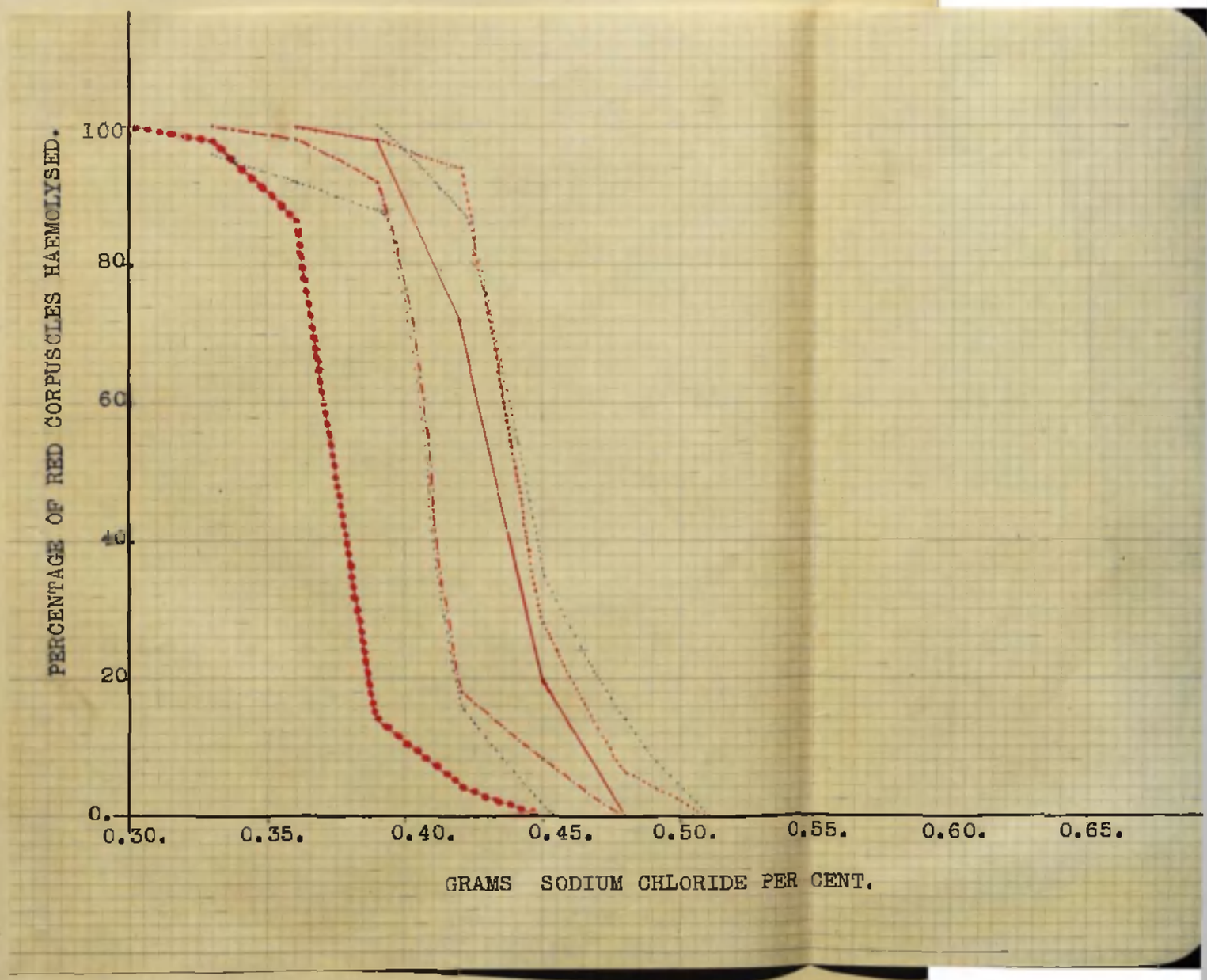


Fig. 7.

..... Fragility Curve of patient's Venous Blood. (Immed.)

——— Fragility Curve of blood with 1-9 3.8% Citrate.

-·-·-·- Fragility Curve of blood in 1-3 Glucose Citrate Saline.

●●●●●●● Fragility Curve of blood in I.M.T. 1-9.

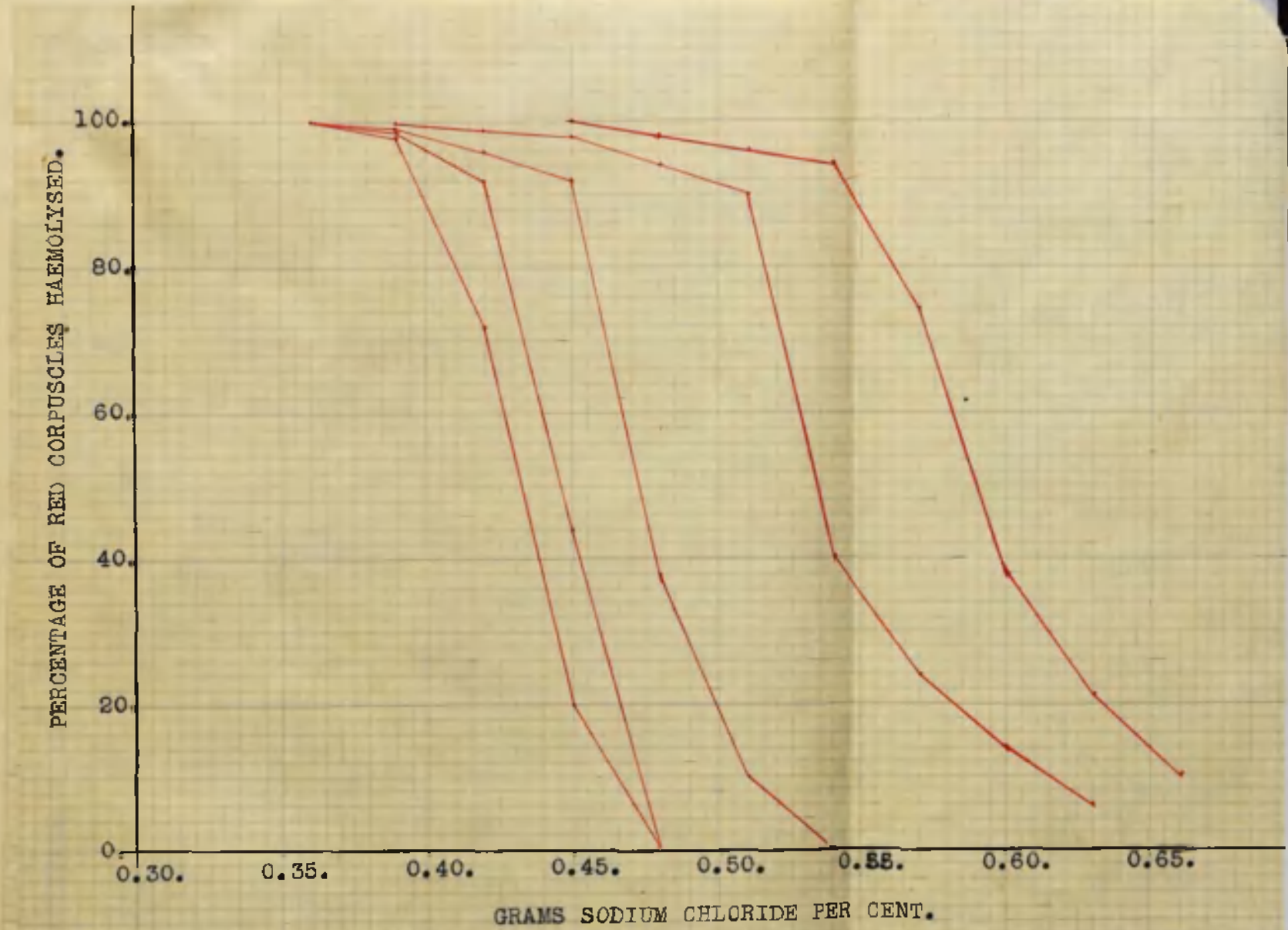
Whitby and Hynes' normal limits are shown in blue

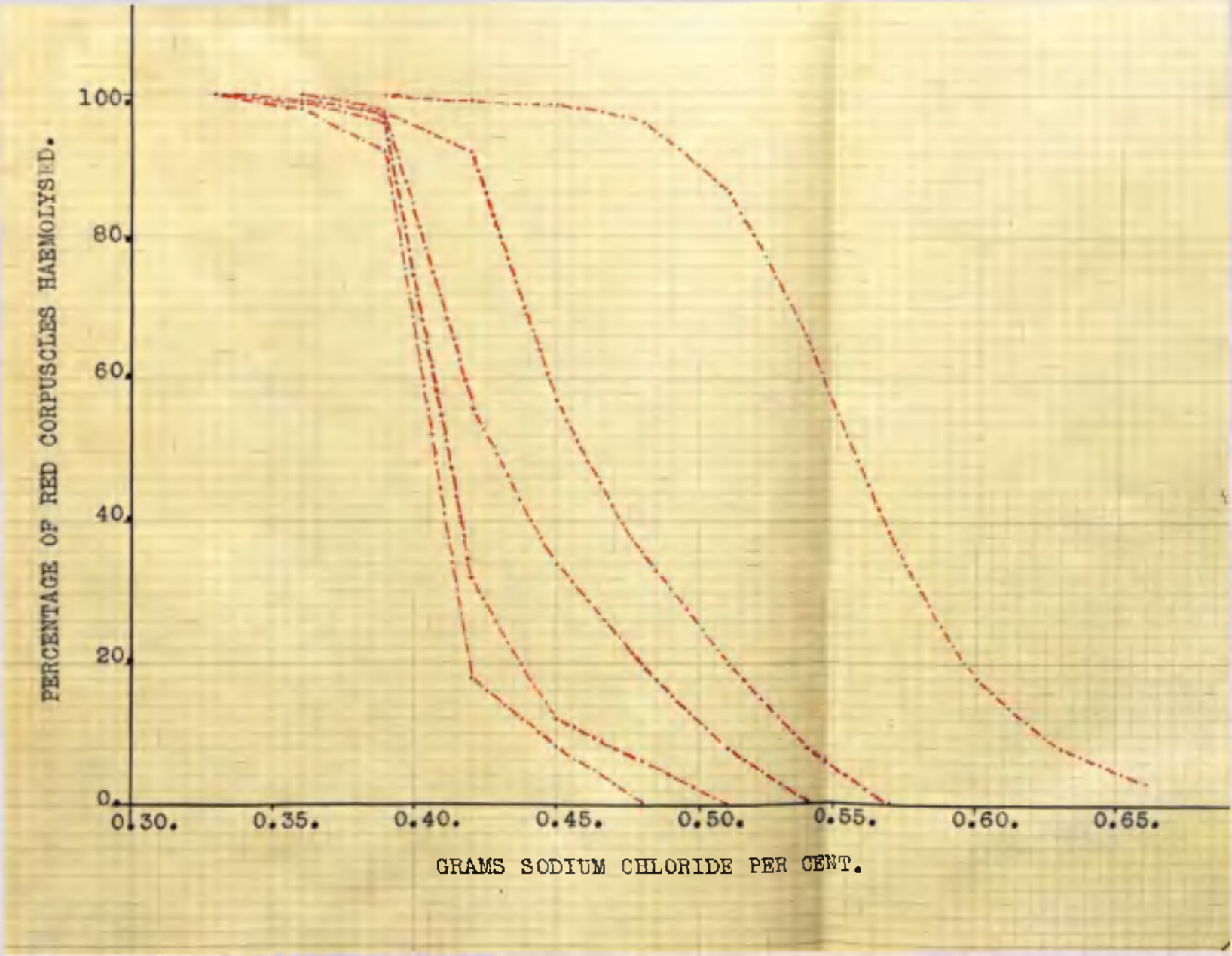
Figs. 8, 9, and 10 show curves taken from these specimens on the 1st, 9th, 13th, and 17th days of storage.

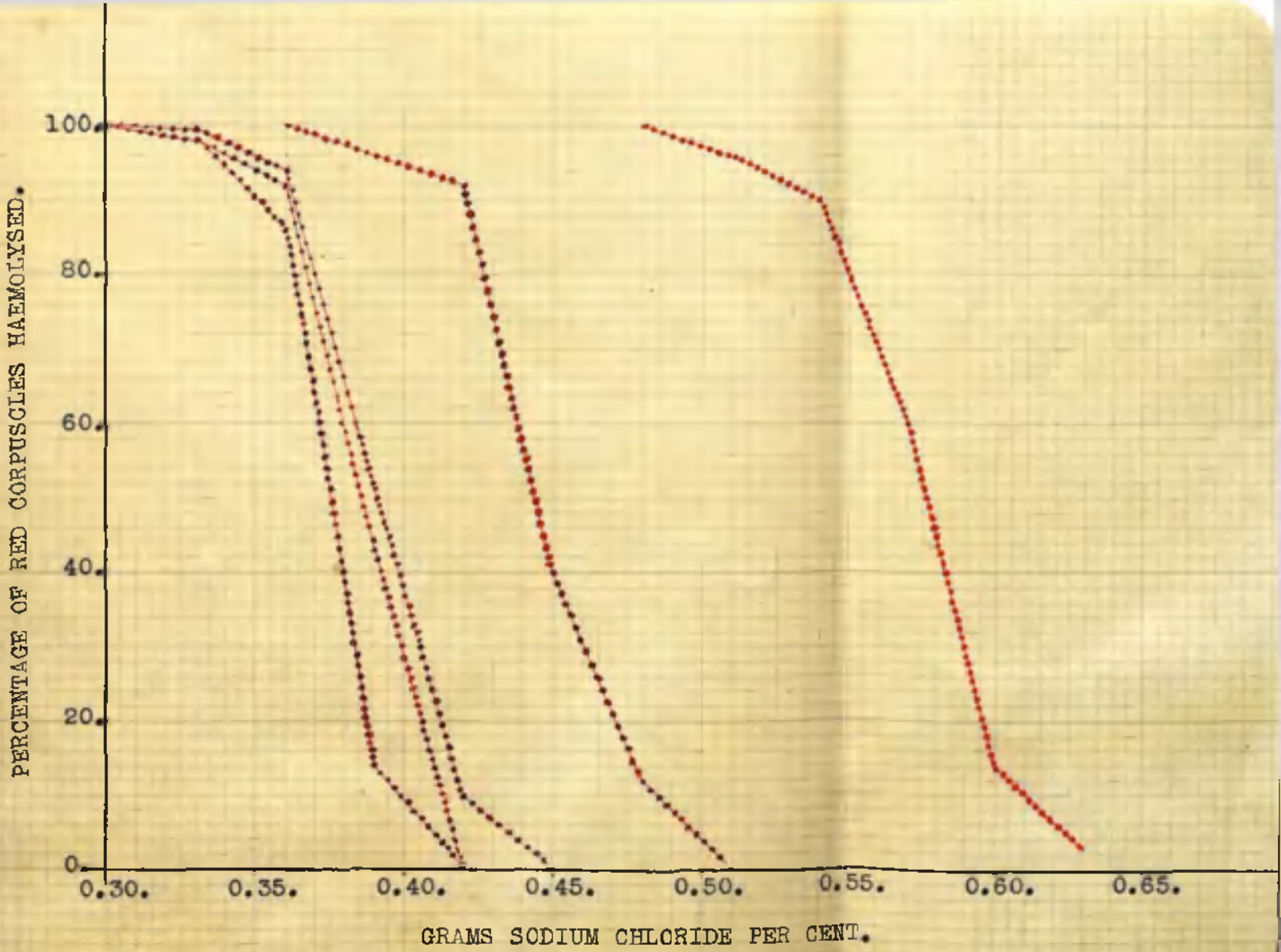
——— Fig 8 ; 1-9 0.9% Citrate Specimen.

-.-.-.-.- Fig 9 ; 1-3 Glucose Citrate Saline Specimen.

●●●●●●● Fig 10 ; 1-9 I.V.T. Specimen.




PERCENTAGE OF RED CORPUSCLES HAEMOLYSED.
100
80
60
40
20
0
0.30.
0.35.
0.40.
0.45.
0.50.
0.55.
0.60.
0.65.
GRAMS SODIUM CHLORIDE PER CENT.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100.
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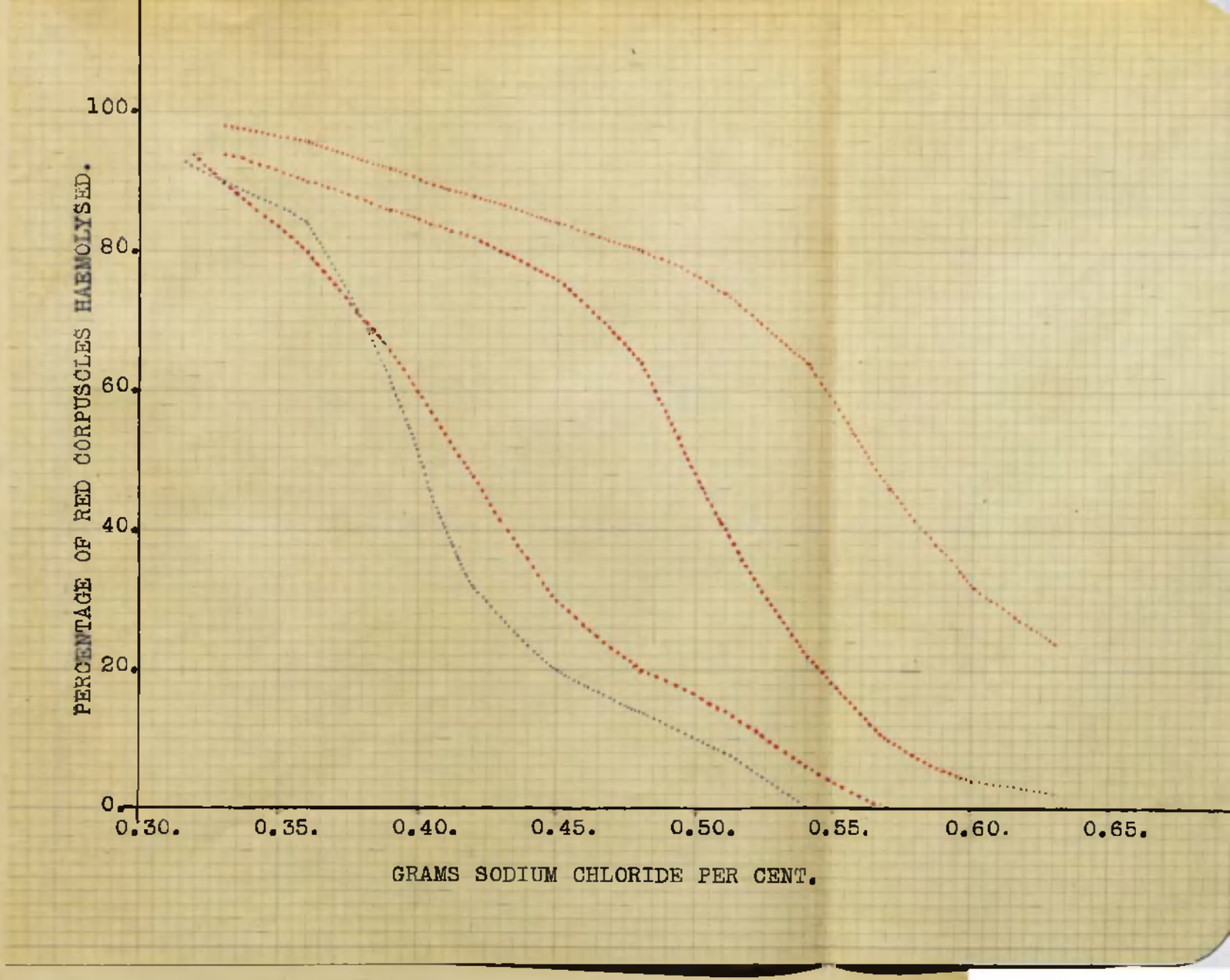

Fig11.

Fig. 11 shows the averages of three specimens of placental blood obtained by syringe from the Umbilical Veins, and preserved in I.M.T. Curves were taken on the 1st, 5th, and tenth days; in all three specimens, considerable haemolysis was present then. The blue dotted line shows Whitby and Mynes average of 20 infants. Other placental samples with different anticoagulants haemolysed even more rapidly, Whitby reports up to 20% haemolysis on mixing placental blood with Citrate . Such early and abundant haemolysis renders the placenta a very poor source of blood unless used comparatively soon after collection, and this, together with the necessity of collecting several bloods of the same group to give one useful transfusion, place it very far behind the healthy donor as a source of blood.

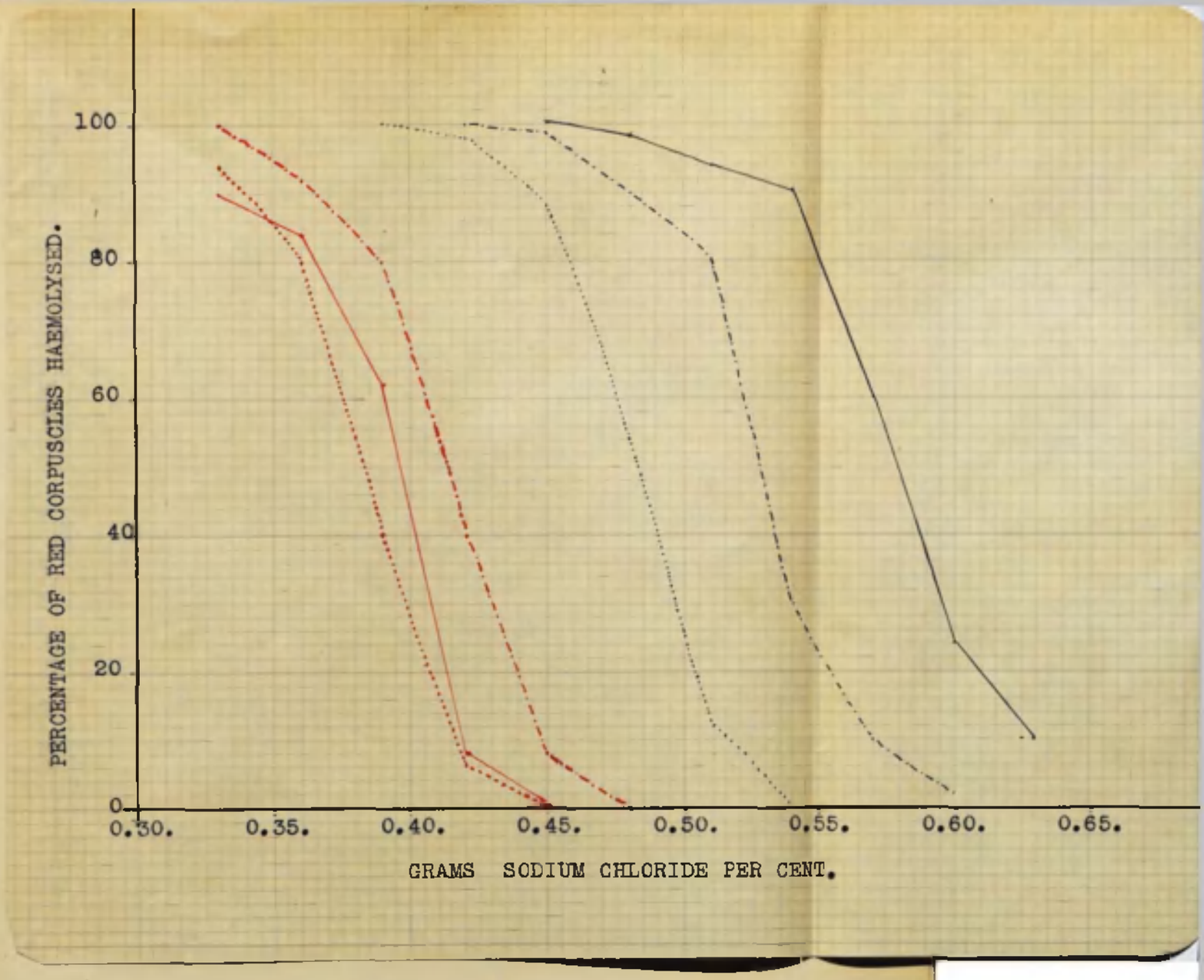

Fig. 12.

This shows average fragility curves taken on the first and fifteenth days from an unselected series of hospital patients. All specimens were stored in Glucose Saline Citrate, and all were oxygenated.

1/ ——— Average of nine children under 14 years.

15 - - - - - - Average of ten women-unselected.

1/15 — . — . — Average of ten men unselected.

These last curves were the result of an examination of 29 blood samples; all were grouped, but that was of no significance nor were any found which varied outside normal limits. Apart from the fact that children's blood decreases in fragility to a greater extent than adults' and males very slightly more than females; nothing outstanding was discovered, but other valuable information was observed in the process.

It became evident early in the investigations of the red cell fragility, that there was a very close relationship between it and haemolysis in stored blood samples. In all, 50 specimens of stored blood considered, irrespective of the donor, as regards age, sex, general health and blood group, irrespective of the anticoagulant used, or the length of time the blood had been stored, if the red corpuscles were not haemolysed by a saline solution of 0.54%, no spontaneous haemolysis occurred, nor could be induced by mechanical violence. The earliest traces of haemolysis occurred when thefragility was equivalent to a saline solution of 0.60%, and at 0.57%, no spontaneous haemolysis occurred in detectable quantity, but mechanical violence, (Centrifugalisation for 10 minutes, and mixture with normal saline,) produced 5-10% haemolysis. Thereafter, as fragility diminished, haemolysis increased, as did susceptibility to mixing. These figures were so remarkably constant that not one specimen varied, though by complete rest of any sample, the rate of haemolysis appeared to be reduced. The red corpuscle fragility, therefore, is of paramount importance when the storage of blood for transfusion purposes is considered, and all means should be taken to enhance and preserve this for the maximum possible time, and further, it is a very sensitive index as to whether a stored specimen of blood is fit for transfusion.

CONCLUSIONS.

1. There is a very close relation between the fragility of the Red Blood Corpuscles and the onset of haemolysis in a stored specimen of blood.

2. This relationship is independant of extraneous factors, and appears to be absolute.

3. All factors which enhance this fragility should therefore be utilised for blood storage.
   a. Oxygenation should be complete.
   b. Small quantities of Magnesium Sulphate enhance fragility.
   c. Glucose partially decelerates the natural decrease in fragility.
   d. Ample dilution of the blood is adviseable.
   e. Adult Female and male blood is preferable to that of children or Placental blood.

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